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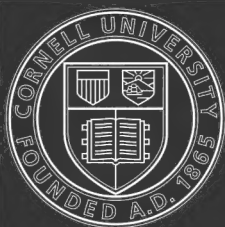
**A STUDY OF THE FACTORS WHICH GOVERN MATING IN
THE HONEY BEE**

BY
GEORGE D. SHAFFER

EAST LANSING, MICHIGAN

1917

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BY GEORGE D. SHAFER.

THE PROJECT.

For the project as entitled above it was proposed to undertake a study which should be directed, in the main, along the following lines of work:

1. An examination of available literature relating to the problem.
2. A study of the primary and secondary reproductive organs of the drone and the queen, with respect to relationships of the organs to each other—in the case of the primary organs, including a histological as well as an anatomical study where that seemed advisable.
3. An attempt to discover mating habits by observations under field or apiarian conditions or by study after resorting to devices which modify those conditions.
4. Certain experimental mating tests, designed in such conformity with the instincts and other factors found to govern natural mating, as might possibly lead to some practical method of control—i. e., a method of mating a selected drone with a certain queen.

These lines of work have not been followed out fully to the author's satisfaction, but it seems best to report, at this time, some results obtained during the past season—1916.

The facts are now well known that, normally, the virgin queen meets the drone in the air on her "mating flight" and that on returning to her hive from that flight, the successfully mated queen bears a part of the torn drone organ in her vulva. Huber* has been credited with having first related these facts, and his description shows that the discovery was original with himself and his assistant. His little book figured, particularly, one part of the drone organ as lost in commerce with the queen (see his fig. 7, Plate II). His figure represents the "bulb" shown herewith in Fig. 4, B. Von Siebold,† also, 1853, learned by direct examination that "those definitely formed parts in the vagina of the queen *just mated*‡ were nothing but the torn copulative organs of a male bee (drone)," but neither he nor Huber carried their studies in this connection further, and apparently no one has recorded any attempt to do so, up to this time. Early in the work on this project, it occurred to the writer that a careful examination of the torn male copu-

*Appendix, "New Observations on the Natural History of Bees," by Francis Huber, 1821 (Translation)—Langstroth in "Hive and Honey-Bee," (1862) says in a footnote that Posel and Jansha were acquainted with certain of these facts as early as 1784.

†"A True Parthenogenesis in Moths and Bees," by Carl T. E. Von Siebold, 1857, (Trans. by W. S. Dallas).

‡Italics inserted to make the context clear.

latory organ in the vulva of the newly mated queen might give positive evidence of the position assumed by the queen and drone relative to each other in the moment of coition. The importance of gaining positive knowledge in this connection is evident. Before attempting to intercept queens returning from the mating flight in order to make the examination just suggested, however, it was only natural to undertake a careful first hand review of the structural characters of the reproductive organs in the queen and drone—especially of the copulatory organs in the drone. This report has to do, therefore, more particularly with a study of the male and female reproductive organs and with the relation of these organs in sexual union as demonstrating the relative position assumed by the drone and queen in that act.

STRUCTURAL CHARACTERS OF THE REPRODUCTIVE ORGANS.

The structural features of the organs of both sexes of the honey-bee have been studied, described, and illustrated by more than one student. Swammerdam's drawings of the reproductive organs of the queen and drone, published in "The Book of Nature," were the first illustrations to exhibit the essential gross characters of those organs. Later, Cheshire (1886) in the first volume of his "Bees and Bee-Keeping," published illustrations of both the male and female organs of the hive-bee. In 1910, Snodgrass, Tech. Bulletin No. 18, U. S. Dept. of Agr., Bureau of Entomology, has also given original drawings of the reproductive organs in the queen and in the drone honey-bee. Attention is called to the drawings of these three workers in particular because their illustrations of the organs mentioned have been accepted and copied in manuals and books on bees in general use today.* A few observations made in the study carried on in this connection should, perhaps, be recorded.

a. ORGANS OF THE QUEEN.

Cheshire has omitted one of the glandular vessels emptying into the base of the sting (see Snodgrass—B. gl., *Alkaline gland of the Sting*) in his Figure 42 entitled "Ovaries of the Queen, etc.," p. 213, Vol. I of "Bees and Bee-Keeping." Also he has represented the poison sac as emptying into the base of the sting on the ventral side of the vagina, or common oviduct, in the same figure which is designated an "under side" view. In the queen bee, the base of the sting and the duct emptying into it from the poison sac lie dorsad (instead of ventrad) to the *bursa copulatrix* and the *vagina*. Figure 57 named "Reproductive organs, sting, and poison glands of the queen, dorsal view," as given by Snodgrass in the reference cited, represents the organs in their true relation. Swammerdam's drawing of the ovaries of the queen, etc., omits the alkaline gland of the sting mentioned above, but the other structures and connections are faithfully represented as they appear in a dorsal view where the muscles at the base of the sting have been separated along the middle line and bent to each side. In a dorsal view, these muscles of the sting very often almost or entirely hide the pair of little side pockets at the posterior end of the vagina—i. e., the pockets

*See the footnote on page 10.

which, together with the posterior end of the vagina itself, form the structure commonly named the bursa copulatrix, Fig. 5. The drawings of Snodgrass and Swammerdam just mentioned, however, show clearly the relation of the large pair of ovaries and the pair of oviducts leading into the common median vessel or vagina—also the globular *seminal receptacle*, or *spermatheca*, with its attendant white accessory glands. The common duct of these little glands passes around underneath the spermatheca, where, in conjunction with the latter, it connects with the upper end of the short spermathecal duct which, in turn, opens through the dorsal wall of the vagina very close to its anterior end. Swammerdam has represented the spermatheca pushed to one side in an attempt to show its duct connecting with the dorsal part of the vagina.

b. ORGANS OF THE DRONE.

Snodgrass has given the most instructive and accurate illustration of the reproductive organs of the drone, natural position, dorsal view, in his Fig. 56, A. Briefly, they consist of the *penis*, the *ejaculatory duct* and a set of paired organs—the *testes*, the *vasa deferentia*, the *vesiculæ seminales* and, finally, the *accessory mucous glands*. The latter are connected by the slender ejaculatory duct with the large invaginated penis which lies entirely within the abdomen along the median line between the paired organs. Of this latter set of organs, the accessory mucous glands are the larger. They have the form of two long sacs, slightly constricted near the middle, having the blunt closed ends turned anteriorly. The narrowed posterior ends of the glands bend toward each other and meet on the median line close above the base of the penis at the extreme caudal end of the body. The walls of these glands, at the blunt rounded ends, are comparatively thin (Fig. 6). Beginning at the region of the constriction, however, the walls gradually become much thicker posteriorly, due principally to the presence of a greater number of muscle fibres. In the region of greatest muscularity, the fibres are arranged more or less regularly into three layers. Figs. 7 and 8 are photomicrographs of a cross section made about one-fourth the length of the gland from its posterior end. They show on the outside a layer of longitudinal fibres; immediately within that, circularly arranged fibres; and, finally, within that next to the glandular cells lining the lumen, a layer of longitudinal fibres marked "c." The lumen of the gland is usually packed to distention with a white granular homogeneous fluid. The active spermatozoa originate in the pair of testes and pass through the coiled vasa deferentia into the long narrow, cylinder-like, curved *vesiculæ seminales* or seminal vesicles there to be stored in quantity. The vesicle walls are muscular throughout their length, and of about uniform thickness. Lying upon the basement membrane of the cells lining the lumen of each vesicle (Figs. 13 and 14) is a layer of circularly arranged muscle fibres which are cut lengthwise in cross sections of the organ itself. Next outside the circular layer is a somewhat thicker layer of longitudinal fibres, and outside of that is an envelope consisting mostly of loose circularly arranged connective tissue fibres and muscle fibres interwoven with many small tracheae. The right and left seminal vesicles each connect by short bent ducts with the narrowed bases of the cor-

responding accessory glands. These two glands empty ventrally, on the median plane, into the ejaculatory duct, which passes at once dorsally to the left and forward in a broad arch to where it joins the anterior end of the resting penis. Snodgrass has given, also, at "E" in his Fig. 56 (reference) an excellent side view of the resting invaginated copulatory organ showing its basal pouches (marked "ZZ" in his figure) unfolded and lifted up. This organ, as well as the ejaculatory duct, is essentially a chitinous tube surrounded by its layer of hypodermal cells. Externally, the tube opens by a wide crumpled slit on the median line far toward the posterior ventral side of the drone's abdomen. In the resting condition the *genital slit* is entirely hidden by the fused triangular plates bounding it on the anterior ventral side and by the dorsal *genito-anal cover plate*. At the extreme posterior end (just anterior to the external slit) the penis wall is very thin and from its sides arise the large membranous pouches or *pneumophyses* (zz) already mentioned. Attached about the wrinkled base of the penis even up to the origin of the pneumophyses are many small tracheae the special significance of which will appear later. These tracheae may easily be seen under the binocular microscope in a properly prepared dissection of the drone's abdomen. Likewise, such a dissection, or reference to Snodgrass' drawing or to Plate I of this bulletin, will show how singularly the wall of the copulatory tube is differentiated in the remainder of its length both as to shape and chitin structure. On the ventral wall just anterior to the bases of the pneumophyses is an oblong, somewhat quadrangular, thick chitinous plate (Fig. 3, xx). Immediately anterior to this plate is a series composed of three narrow *cross-stripped chitinous ridges* and four more weakly chitinized ones with corresponding creases—the whole set appearing, at first sight, somewhat as a broken or interrupted spiral band (vv, Fig. 3). On the dorsal wall opposite the anterior end of the *ventral quadrangular plate* is a *triangular plate* of chitin (ww) having its broadest or basal portion directed backward. Both of the plates and the stronger cross-stripped chitin ridges are clothed with spinules or hairs inside the resting tube. The extreme forward end of the copulation tube is greatly enlarged into a pear-shaped *bulb* (B, Fig. 3) which goes over in the ejaculatory duct (EjD) at its anterior point. In the dorsal wall of the bulb is a pair of *symmetrical chitinous plates* (tt) one lying on either side of the median line. They are widest at their anterior ends and taper to slender, *parallel prongs* (P) at the posterior ends. Also each plate bears posteriorly by a thin arm a little *chitin-wing* (wc) on the corresponding side of the bulb. The hypodermis of almost the whole dorsal wall of the bulb presents a double longitudinal fold, which gives rise not only to the symmetrical plates and little chitin wings already described, but also to a large *elastic thickening* (named by Snodgrass) of tough gelatinous-like consistency. This *elastic thickening* is shown in cross section after eversion at "ss" in Fig. 9 and in surface view Fig. 4. All the thinner portions of the penis wall are whitish in appearance, while the heavier chitinized portions (i. e., plates) are yellowish brown to dark brown. Finally, arising from the dorsal side of the tube immediately posterior to the bulb is a thin-walled *doubly pinnate*-

lobed projection, (ww, Fig. 3) the lobes of which are turned toward the bulb.

It will thus be seen that the dorsal and ventral walls of the penis tube bear distinguishing characters. This is equally true after the organ becomes evaginated, as it does in the act of copulation. In that act it is generally believed that the whole tube of the penis turns out through the genital slit into the vagina of the queen. That is, the pneumophyses evaginate into the pouches of the bursa copulatrix—the pinnate lobe and the bulb everting in turn and pressing deeper within the vagina. It is well known that if a flying, excited drone is caught and held between the thumb and finger or in the hollow of the hand, the copulatory organ will sometimes be suddenly everted with force. The abdomen contracts rigidly to less than half its former size as the organ is expelled, and the insect quickly dies. Also, as a drone is held between the thumb and finger, pressure on the abdomen (from before backward) may often be used successfully to fully expel the organ—especially when the tracheal system is well filled with air. Cheshire, at "E" Fig. 41 (citation) and Michaëlis,* Plate XXVI, have both given illustrations of the drone organ in the fully extruded condition. In order to help show the relation of the structural parts in the extruded penis, an illustration of the same is also given herewith, Fig. 4. Pressure, brought to bear upon the contents of the drone's abdomen through the contraction of the abdominal wall muscles, normally starts the process of evagination. As the abdomen contracts, the confined air in the vesicles of the tracheal system becomes compressed†; the blood in the body cavity, however, moves freely to the area of least resistance—to the base of the penis. In that moment the crumpled tubular penis wall, next the genital slit, is pushed through the slit and swells out behind—expanded with blood. Eversion has begun, and with it the small tracheae at the base of the penis are stretched lengthwise to the limit. They yield and break as the large, ventral, quadrangular, spine-covered plate rolls out and, thus, they release the compressed air of the tracheal vesicles within the evaginating fold of the partly protruded penis. Instantly, the pneumophyses dart forth and curve downward in the form of two hollow white horns, filled with *air* and *blood*. The cross striped ventral ridges and the dorsal triangular plate appear. Each everting portion of the penis draws after it the next anterior portion down through the part of the tube already evaginated until, finally, the doubly pinnate-lobed appendage‡ begins to show. Then the bulb snaps out and the process of evagination stops at the point where the former anterior end of the bulb—now the distal end of the extruded organ—connects with the narrow ejaculatory duct. The opening of that duct may be seen in the upturned tip of the fully extruded penis (o, Fig 4), and from there through the lumen of that organ the slender ejaculatory duct itself may be traced to where it still attaches to the accessory glands at the posterior end of the abdomen—sometimes the ends of the paired organs are found partially forced into the distended base of the penis. The position of the ejacu-

*Michaëlis, Georg. Bau und Entwicklung des Männlichen Begattungs—apparates der Honigbiene. Zeitschr. Wiss. Zool. LXVII. 1900, pp. 439-60.

†Djathchenko, Sophie, 1906 in Ann. de L'Institut Agron. de Moscou, figures the closing apparatus of the Stigmatal tracheae of the bee.

‡This appendage evaginates normally just as the bulb snaps.

latory duct in the fully evaginated male organ is indicated by dotted lines in Fig. 4. Figs. 12 and 9 show the ejaculatory duct in longitudinal and in cross sections of the evaginated organ, respectively. Michaëlis (page 456, citation) understood this position of the duct in the extended organ, but he did not figure it. The *elastic thickening*, which gave a bulbous or convex form to the dorsal side of the penis bulb in its resting condition, now presents itself as a concavity or cup in that same side of the everted bulb (c. Fig. 4). Above the outer or distal edge of the cup is the opening from the ejaculatory duct (o, Fig. 4), and before the opposite edge stands the fully expanded doubly-pinnate lobe facing toward the mouth of the duct, while from the edges of the cup on either side of the pinnate lobe arise the pair of little *chitin wings* (wc). The plates which bear these little wings lie partly covered by the elastic thickening, now, with their slender free prongs (p, Fig. 4) directed proximally.

The above description of the extruded drone organ and of the act of extrusion does not agree in certain essentials with the description given by Cheshire. Microscopic sections of the ejaculatory duct show that its walls are *not* muscular (Fig. 10). Both longitudinal and circular muscles occur in the walls of the seminal vesicles and in the walls of the posterior portions of the accessory glands, however; but these muscles serve another purpose* and are not in a position to assist in the evagination of the penis. On page 202, Vol. II, Cheshire states that "The bean, and the remaining parts, from o to m, are surrounded by a membranous sheath, which remains intact after the expulsion mentioned." In accordance with that statement, his drawing, A, Fig. 41, p. 199, shows two walls from "o to a"—i.e., the bulb and the remainder of the resting copulation tube is represented as lying within a sheath which, itself, connects with the wall of the ejaculatory duct. Now, dissections and microtome sections of the resting male organ show the wall to be single (see Fig. 11), and, indeed, the wall of the ejaculatory duct goes over into the wall of the bulb. Unfortunately, the drawing A, Fig. 41 of Cheshire, mentioned above, has been copied as late as 1908 in Berlese "Gli Insetti," Vol. I, p. 864.

In case of almost all the dissections made of drones, taken before the hive entrance, the bulb of the resting sexual organ was found to not contain spermatozoa in quantity, and no "spermatophore" was found. Cheshire's *spermatophore*† as described on page 203, Vol. II appears, in reality, to possibly be the *elastic thickening* of the wall of the resting bulb. In the few instances in which spermatozoa were present in the dissected resting bulb, it is believed that they were forced there by incomplete muscular contraction of the "paired organs" during the process of opening the drones' abdomens. Several times, while studying the organs of a drone that had been opened under water, the paired organs contracted somewhat so that—through the partially transparent wall of the ejaculatory duct—the granular sperm fluid could be seen as it was forced from the accessory glands. All of this leads to the belief that the pair of seminal vesicles and the posterior

*See line 4, p. 15; also, last part of footnote p. 11.

†Packard in "A Text-book of Entomology," p. 500, quotes Leuckart as describing a spermatophore, a sort of seminal cartridge, in case of the honey-bee. I have not seen Leuckart's paper. Lang, in his Comparative Anatomy, gives a figure of the drone's reproductive organs taken from Leuckart.

ends of the accessory glands (*not* the "bean" or bulb)* are the regular, normal, and final reservoir for spermatozoa in drones able to fly.

Since the bulb was regularly found to be empty, rather than filled with spermatozoa or any definitely arranged compact spermatophore, the question may well arise as to the actual reason for existence of the bulb-cavity and the swollen ventral wall of the same. It is possible that during the final marriage flight the bulb-cavity may receive some spermatozoa; that may not be determined—however, a necessary and actual use of the cavity may be observed by holding a drone and carefully watching the large dorsal elastic thickening and the accompanying dorsal plates as the bulb is made to pass slowly through the process of eversion. It will be seen that the thickening and plates together, although elastic, can not make a short bend during evagination as does the thinner portions of the wall, but that in effect, they must somersault or swing, in a body, around the "prongs" of the plates as a pivot within the cavity afforded by the expanded ventral wall of the bulb. In other words, the large elastic thickening of the bulb with the pair of accompanying plates *could not evert* if the thinner opposite wall of the bulb were not correspondingly enlarged. The use of the elastic thickening and the pair of bulb-plates, themselves, will appear later (see page 15).

When the characters of the reproductive organs of the drone and queen had thus been reviewed carefully and fixed in mind, measures were taken to observe queens leaving the hive on the mating flight and to intercept them on their return in order that the exact orientation of the torn male organ in the vagina of the queen might be studied.

POSITION ASSUMED BY THE QUEEN AND DRONE IN COITION, ON THE MATING FLIGHT.

The queens used in making observations on the "mating flight" were obtained directly from nursery cages kept in a hive body above the brood chamber of a strong colony, and separated from it only by a queen excluder. These queens were introduced into hive nuclei, containing three to five regular Langstroth frames, either by the introducing cage, or by the smoke method. Over the entrance to each nucleus was kept an Alley's Improved Queen and Drone Trap—the trap entrance at the back stopped to a small opening. The traps were used in order that the virgins might not take flight except when the nucleus was under observation. Other work made it impossible to watch the nuclei *all the time* during that period of the day when the virgins were most apt to attempt to fly. The plan, therefore, made it possible to conserve virgins for those periods when observations could be made and in that way much time was saved. Any immediate or recent movement of the queen from the nucleus into the trap was quickly made known by a certain excitement of the workers in the trap-entrance and at

*Two other considerations would make it seem unlikely that the cavity of the bulb should be regarded as the final reservoir for spermatozoa before their ejection into the vagina. First, the large amount of sperm fluid found in the vagina of the "newly mated" queens examined (as explained later, p. 12) seemed much more than could be contained by the space of the bulb-cavity. Second, no such muscles as are found in the walls of the seminal vesicles and accessory glands (Figs. 13, and 7 and 8) would be needed to pass the spermatozoa along *slowly* to the "bulb," there to be stored. The heavy musculature in the walls of these paired organs indicates a function of squirting the sperm fluid through the ejaculatory duct with considerable force.

such moments she was usually eager to fly when quietly released from the trap. If, on the other hand, the queen had been in the trap for some time, having come out when the nucleus was not under observation, her presence was almost always marked by a small cluster of workers. A flight did not often occur in the latter case, and as a rule it was found better to simply allow her to run back into the nucleus—no difficulty was experienced with any queen when the trap was momentarily removed and she was placed in the nucleus entrance.

Of course, when a virgin was once released and took wing, watch had to be kept for her return home, but the trap would then prevent her entry until she could be captured for study, or allowed to "run in" according to the wish of the observer.

Nine virgins were observed in eighteen flights. Five of the nine were lost, but the other four were captured "newly mated," with the torn drone organ present in the vulva, before their respective nuclei. The vaginae of two* of the four queens were dissected open carefully in order to examine the part of the drone organ present and in both cases the bulb only, of that organ, was found. A study and comparison of the two showed that both bulbs had been everted. Studied with reference to orientation in the vagina, the gelatinous-like thickening forming a concavity or cup, in each case, faced dorsalward and anteriorly. The convex side of the bulb was turned ventrally and slightly toward the mouth of the vagina. The "prongs" of the two symmetrical bulb-plates were directed posteriorly and dorsally—their curved points against the dorsal wall of the vagina. Almost all the membranous parts had been stripped from the gelatinous-like thickening and chitin plates of one of these bulbs. In case of the other, however, the penis had been torn off between the pinnate-lobed appendage and the bulb, while the ejaculatory duct also had been torn away at its point of attachment with the latter. The part of each bulb which faced up and anteriorly, toward the inside of the vagina (i. e., the cupped part of the bulb), was covered with the thickish, white, coagulated sperm-fluid. This fluid, in fact, packed all the part of the vagina anterior to the bulb, and in one queen the oviducts also were quite distended with it. Figs. 17 and 18 show cross sections of one oviduct of this queen through the portion most widely distended with the sperm-fluid. Both spermatozoa and the granular secretion from the accessory mucous glands are shown to be present mixed together. The seminal receptacle of this same queen was sectioned and studied. But very few of the spermatozoa had gained access to it†—showing that the receptacle was not filled by the first discharge of the spermatie fluid into the vagina in this case, at least.

The other two of the four queens referred to in this connection were not dissected. It was determined for both, however, that the everted bulb was the part of the male organ that showed as a "white pellicle" in the vagina. One of these queens was caught upon her return trip and held, while, with a pair of forceps, the bulb was gently worked loose by pushing downward until it could be slipped out of the vulva. As in the former two queens, the concave part of the bulb was turned

*Both killed in hot chloral hydrate (water) solution—1 to 20.

†The queen had been out on her mating flight 22 minutes when she was caught before her nucleus, mated;—she was then killed within five minutes.

dorsalward and forward, facing inside the vagina—i. e., toward the attachment of the seminal receptacle (spermatheca).

The fourth queen had been gone only 15 minutes when she returned mated. In her case it was not found necessary to remove the bulb from the vagina in order to determine that its orientation was the same as in the three instances already described, and that it also was everted. As soon as the bulb had been examined by turning it a little from side to side with the forceps, this queen was placed at the entrance to her nucleus and allowed to run in. Three minutes later the nucleus was opened;—it was the purpose to determine, if possible, what disposition would be made of the bulb which had been left in the vulva. Workers were found to be following the queen about very attentively, some of them nipping at the torn male organ which seemed now about half out of the vaginal opening. Very soon the queen passed around on another frame, and when caught sight of again, the white penis-bulb was lying on the hive bottom just beneath her. Whether a worker actually pulled the bulb from the queen was not observed, but she was free from it within scarcely more than five minutes after she entered the nucleus. Undoubtedly this bulb had been loosened a little in the vagina by the forceps during the examination mentioned; but it was noticed also that the elastic gelatinous-like thickening was slightly shriveled when the bulb was picked up from the hive bottom. In order to determine whether this mating would really prove successful, the queen's wings were clipped and she was then introduced into a strong nucleus, the hive entrance of which was covered with a queen and drone trap. She was found out in the trap only once, three days after the introduction. On the twenty-first day she had three frames of sealed worker-brood besides other larvae and eggs. It would seem almost certain, therefore, that spermatozoa had been stored in the seminal receptacle.

If we consider the four cases just described together, now, the practically similar statements with respect to orientation of the bulbs in the vaginae will appear most significant only when considered with the determination (stated in each instance) that the bulbs were fully everted. This determination itself rests upon evidence afforded by two observed conditions which should perhaps be clearly repeated in this connection:

First, The pair of symmetrical bulb-plates, within the vagina, were placed so that their "slender prongs" pointed dorsally and toward the opening of the vagina in each case—i. e., toward the position that the base of the copulatory organ must have occupied before it was torn away, and that relative arrangement of the plates can occur only in the fully evaginated organ (see Fig. 4)—not in the partially evaginated organ.

Second, The concave surface of the gelatinous-like thickening was *not* enclosed by the membranous portion of the bulb-wall, but the convex side of the thickening *was* covered by that membrane (except in the instance mentioned when the latter was almost entirely torn away)—a condition, again, which can be true only of the fully evaginated organ.

One other newly mated queen, with male organ attached, was caught

and properly preserved* for me by Mr. M. H. Hunt, a queen breeder of Redford, Michigan. In this case the evaginated penis seemed to have been torn off in the region between the cross-striped chitinous ridges and the quadrangular plate. The somewhat shriveled, torn end of the organ projected backward from the mouth of the queen's vagina, and was pierced from above by the sting. Fig. 19 is a photograph of this queen's abdomen, showing the projecting male organ and the tip of the sting sticking through at Stn. Near the tip of the sting, on the side of the organ corresponding with the ventral side of the queen, there appears what certainly seems to be the cross-striped chitinous ridges. It will be remembered that these ridges are on the same side of the evaginated penis as the convex portion of the bulb (see Fig. 4). Microscopic sections, made longitudinally in a dorso-ventral direction through the entire abdomen of this queen, were attempted in order to show the drone organ in place within the vagina. Unfortunately, the most valuable part of these sections was ruined so that they do not give direct evidence in regard to the orientation of the bulb. The cross-striped chitinous ridges, however, indicate that the copulatory organ, in this case also, was placed in the vagina exactly as was determined in the four instances where the entire evidence had to be gained from the penis bulb alone, since in those instances the convex side of the evaginated bulb was turned *ventrally* (and outwardly) relative to the queen's vagina.

It has already been given as the general belief that the penis tube turns out or everts through the genital slit directly into the vagina of the queen. The pouches of the bursa copulatrix of the queen, the manner of extrusion of the drone organ, its size and its irregularity in form, as well as the helplessness of the drone almost immediately after extrusion, make it practically certain that this view is the correct one. In accordance with this view it would be necessary for the genital slit of the drone to be placed close to the outer vaginal opening before extrusion of the male organ. That being the case, evidence has already been presented by which it is possible to decide definitely the position assumed by the queen and drone relative to each other during coition.

The evidence from the five instances described shows that each drone presented himself in such a manner as enabled him to place the male organ within the vagina with the dorsal side of the former against the dorsal side of the latter—with the cup of the evaginated penis-bulb and the mouth of the ejaculatory duct directed upward and inward toward the duct-opening of the seminal receptacle. In order to ever accomplish such a result there seems to be but one possible position which the drone and queen may assume, relative to each other, at the moment of coition—they must meet and clasp face to face. In that position, the abdomens press together, naturally. The grooved tip of the fused plates bordering the drone's genital slit passes easily through the "sting notch" (sn, Fig. 15) to push the sting dorsalward and force the queen's genito-anal cover shields apart. Forthwith, we may imagine, the drone's abdomen might contract and start evagination of the penis tube. This, then, would cause the large "quadrangular plate" to press ventrally until it opened the mouth of the bursa copulatrix and allowed the pneumophyses to dart

*Killed in boiling hot picric alcohol, made up by mixing a saturated solution of Picric acid in 95% alcohol with distilled water, equal parts.

into the "side pouches." Thus, might the whole process continue until the elastic cup of the bulb itself snapped over in its proper position and directed the mouth of the ejaculatory duct toward the inner cavity of the vagina. It is at that moment, most probably, that the muscular walled seminal vesicles and accessory glands contract and send their contents through the ejaculatory duct in such quantity as to fill the whole inner vagina and distend its walls. Also, it must be then that the winged, concave, elastic bulb-thickening (assisted dorsally by the bilobed appendage*) performs its principal function as an effective stopper to hold the sperm-fluid within the vagina. When the drone falls back helpless, and the copulatory tube becomes torn, the thinner portions of the tube walls collapse; but the thick elastic part of the bulb remains firm—the "slender prongs" of the bulb-plates holding securely against the dorsal wall of the vagina—until after the gelatinous-like portion begins to shrivel, when the whole bulb becomes dislodged.

In looking up references upon the mating of queen honey bees, two quite definite observations were found recorded—both agreeing with the determination given here as to the position necessarily assumed by the queen and drone in sexual union. The essential facts of these two records follow:

Mr. S. A. Shuck (Am. Bee J., 1882, p. 789) records that he fastened a virgin five days old on a thread ten feet long. He then secured the thread at the end of an eighteen-foot pole which he raised in his bee yard at about 2:30 o'clock on an afternoon when numerous drones were flying. The drones chased the queen at intervals, and finally one of them caught her apparently face to face for a few seconds only; the drone quickly swung back, head downward, snapped and fell to the ground. The queen then bore the "evidence of her mating."

Mr. E. A. Pratt (Gleanings in Bee Culture, and A B C and X Y Z of Bee Culture by Root) says: "On June 21, 1888, I saw this mating take place. The queen issued from the hive, took two circles and came within five feet of my face, and was there met by a drone. They seemed to face each other, clinging by their fore legs, their bodies being perpendicular, and in this shape flew from my sight. * * * * *"

ATTEMPTS TO CONTROL EITHER MATING OR "FERTILIZATION" OF THE QUEEN HONEY BEE.

The usual and normal method of mating of the queen honey bee is on the wing and in the open. Many bee-keepers have asserted that it can occur only in that way. The bee journals, however, record many instances of successful attempts to control either the mating of queens, or their fertilization, through confinement, or by some hand method. None of these attempts have yet proven practical. Discredit or doubt has been placed upon some of the claims; and rather generally, they have been ignored. Nevertheless, many of the recorded attempts seem to deserve consideration; and it may, perhaps, be profitable (without making a complete review) to briefly classify the means used in the case of

*The fans of the bilobed appendage appear to have the purpose of pushing out to fill all irregularities around the "prongs" of the bulb-plates between the "little wings" in order to prevent a leak of the sperm-fluid there when the latter is sent in quantity, with much force, into the anterior vaginal cavity. As has been pointed out, page 10, the bilobed appendage and its little flaps are directed toward the bulb as they evert.

twenty-four instances taken, mostly, from Gleanings in Bee Culture and the American Bee Journal.

I. Classification of Certain Recorded Attempts to Control Mating:

a. Instances of mating during confinement in one way or another.*

1. Workers allowed to fly in the open, but queens and drones confined by "Queen and Drone Excluders" so that they could fly only in a large enclosure of screen, cloth-netting, or glass. McLain, *Am. Bee J.*, Vol. 24, 1888, p. 487—6 queens successful; Davitt, *Adv. Bee Culture* by Hutchison, 1905—100 queens in one summer; Church, *Gleanings in Bee Culture*, Vol. 34, No. 24—1 queen.

2. Workers, queens and drones all confined in greenhouse, or in large tent. McLain, *Am. Bee J.*, Vol. 23, 1887—1 queen out of 6—later, 3 queens out of 6; Hohenshell, *Am. Bee J.*, Vol. 23, 1887—2 queens.

3. Queen and drones confined in a small nucleus above a colony by screen or by a Queen and Drone Excluding honey-board, but allowed to fly in a small glass or cloth covered mating box placed over the nucleus. Cramer, *Am. Bee J.*, Vol. 17, p. 19—2 queens; Filbert, *Am. Bee J.*, Vol. 16—1 queen; Hasbrouck, *Am. Bee J.*, Vol. 11, 1873—many queens fertilized, also had many failures; Arms, *Am. Bee J.*, 1881, p. 50—has had good satisfaction.

4. Virgin queen and drone, without workers, confined together at right time in a small mating chamber—glass or cloth-covered top. Hasbrouck, *Am. Bee J.*, Vol. 15, p. 519—3 queens; Fincastle, *Am. Bee J.*, XVII, 1881—5 queens; Brown, *Gleanings in Bee Culture*, 1884, p. 674—1 queen.

5. Virgins not able to fly from hive because of imperfect wings or because wings were clipped. Cook, *Gleanings in Bee Culture*, Vol. X, p. 544—1 queen; also *Am. Bee J.*, Vol. 21, p. 154—rarely in confinement in hive; Brown, *Gleanings in Bee Culture*, VIII, p. 33—1 queen; Davis, *Am. Bee J.*, Vol. 15—1 queen; Bagby, *Am. Bee J.*, Vol. 15, p. 553—1 queen.

6. Queen tied to a thread so that she could fly only above the home yard. Shuck, *Am. Bee J.*, 1882, p. 789—1 queen.

b. Instances of "Artificial Fertilization" by hand:

1. By squeezing drops of sperm-fluid from the drone organ into the vulva of the queen. Balch, *Am. Bee J.*, Vol. 23, 1887—had practiced this on queens with defective wings for 15 or 20 years; McLain, *Am. Bee J.*, Vol. 23, and Baldwin reported by McLain—certain of 25 queens.

2. By squeezing contents of seminal vesicles (dissected out) directly into the vulva. McLain, *Am. Bee J.*, Vol. 23—3 queens.

3. By diluting sperm-fluid and using a pipette or syringe to introduce the fluid into the vagina. McLain, *Am. Bee J.*, Vol. 23—6 queens out of 27; Jager and Howard, *Science*, N. S., Vol. XL, No. 1037, p. 720—1 queen.

*The sizes of the confining cages described in the references cited in this outline vary from an enclosure 30 ft. in diameter and 30 ft. high to a 2 quart glass fruit jar.

The A. I. Root Co., is now conducting a mating experiment in a large greenhouse nearly 600 ft. long, 60 ft. wide and 30 ft. high.

Phillips (*Beekeeping* by Phillips, p. 69) has reported attempts at mating, in a large glass covered enclosure, without success.

While none of the methods outlined have been of practical use in queen breeding as yet, the instances of success in certain cases (as claimed) should be sufficient to encourage further trials and study. Indeed, great benefit might be expected from any method that would enable a queen breeder to practice definite selection of the drone as well as the queen in mating merely his "breeding stock."

II. Attempts, at Controlling Mating, Carried Out Under This Project.

Before the close of the season (1916) fourteen virgins were used in numerous attempts to bring about mating with a particular, selected drone in each attempt. The conditions under which these trials were made follow:

1. All the young queens were confined to nuclei of young worker bees, before and after the tests, by Queen and Drone Excluders. Tests were carried on before the window of a small canvass house on the apiary grounds. Also, the virgins' wings were clipped when the test would permit that.

2. Alert drones were selected from those caught (a few at a time) at the hive entrance as they were taking flight, or just returning to the hive. Then, they were kept on wing before the confining window until needed.

3. Virgin queens which were old enough for the mating flight—usually those, 3 to 10 days old, that were attempting a flight were used.

4. The trials were made on hot, sunny days during that time of day when most drones were flying and when it was found that the virgins usually issued for the mating flight—11 a. m., to 3:30 p. m.

5. The attempt was made in every case to bring the queen and drone together face to face.

Two methods of bringing the queen and drone together in the correct position were attempted. By one method each bee was fastened at the end of a fine, elastic wire holder (Fig. 1, p. 19) and then set to humming the wings. The holders permitted such freedom as would induce the insects to keep flying—or trying to fly; yet, the operator might so direct the movements of both queen and drone as to cause them to bump together, on the wing, face to face. Only a comparatively few trials of this kind were carried out. No case of mating was obtained, although the drones often clutched the queens. One queen killed two drones in such attempts.

By the other method the operator endeavored—through squeezing the drone—to evaginate the male organ into the vagina of the queen in the normal position. A queen was held ventral side up between the thumb and forefinger of the left hand; a drone was held dorsal side up, but otherwise in the same manner by the right hand. As the insects were brought face to face, the queen's dorsal and ventral genito-anal cover shields were separated; the tip of the drone's abdomen was then inserted in the opening and his organ evaginated by skillful pressure of the thumb and finger. The task proved to be a difficult one, since a queen is very active with the tip of her abdomen and with her sting. It was necessary, of course, to keep the sting pressed dorsalward dur-

ing the operation, and the drone organ had to be everted at just the right moment—when the sting was slightly protruded—otherwise, the bulb would not press deeply enough into the vagina to hold; and the organ might be prevented from entering the vaginal opening at all. Two different devices, or instruments, for separating the queens' cover-shields and pressing the sting dorsalward were tried.

Fig. 2 shows a block holding a pair of sharp-pointed forceps which have a very thin plate soldered to the lower edge of the right-hand prong, near the tip. With the prongs pressed together, the tip of the forceps could be inserted into the "sting notch" ventral to the sting (the queen being held ventral side up) to force the cover plates apart. A peg in the block allowed the forcep-prongs to open about one-fourth inch. The male organ might then be made to enter *between* the tip of the prongs while the thin plate (a, Fig. 2) held the sting dorsalward. A pair of Bausch and Lomb "Binocular magnifiers" were found most helpful in this connection.

The other device consisted simply of a No. 5 insect pin, with the head bluntly pointed, bent at right angles one-sixteenth of an inch from the head end, and inserted firmly in a block as shown at i, Fig. 2. The bluntly pointed pin-head could be inserted in the "sting notch," and as the queen's cover shields separated, she could be moved so that the pin passed over the sting and held it dorsalward. This device was the simpler of the two, and served the purpose well; however, no matings that proved successful were obtained with either device. In the case of two queens in particular, the male organ was torn off and the inverted bulb was held in the vagina exactly as has been described in normal matings. Later examinations of the queens' seminal receptacles proved that no spermatozoa had been stored in them. One of these queens was kept (before dissection) in her nucleus until she had produced about one-third of two frames of drone brood in worker comb. The brood came out normal, except that the individual drones were small. Living spermatozoa were found in their seminal vesicles. Two other queens with which controlled mating had been attempted, were kept in their respective nuclei until a small amount of drone brood had been started in worker cells. The abdomens of all these "drone laying" queens were somewhat enlarged, and dissection showed a few fully developed eggs in some ovarian tubules, although the ovaries were not as fully enlarged as are those of fertile laying queens. Most of the virgins used in the tests, however, continued to come out into the queen traps, at intervals, in attempts to make mating flights evidently, until they wore themselves out.

Why was it that even the matings which were *apparently* normal in every way proved unsuccessful in storing the seminal receptacles with spermatozoa?

McLain and Baldwin (Am. Bee J., Vol. 23, p. 567) state that in pressing out drones they found only a comparatively few that yielded a fluid seminal secretion resembling albumen. Others gave no secretion, or only a thick curdy supply. It was with the fluid resembling albumen when pressed from the drone organ, that these men claim to have had success. McLain believed that drones yielding practically no fluid, or only a curdy supply, through evagination of the male organ by hand-

pressure, were not able to produce the abundant, more fluid secretion because they had not been properly fed by the worker bees.

Observation of the actions of drones during a honey flow and just after a honey flow can not fail to show that drones must be well fed in order to be rapid, alert and business-like on the wing. Still, with the most promising alert drones, hand pressure usually failed to cause an abundant discharge of seminal fluid exactly like that obtained (for example) when a drone held in the warm hand sometimes performed the act himself. In seeking a reason for this the writer examined the seminal vesicles and accessory glands of a number of select, active drones after they had suffered evagination of the copulatory organ through hand-pressure. Sometimes one or more of the glands and vesicles were found to have been partly crushed—or, pressed far into the everted penis by the side of the ejaculatory duct—usually, without having emptied themselves through the contraction of their own muscular walls. Of course, a small amount of the crushed gland secretion was often forced through the ejaculatory duct, but the pressure stimulus as applied to the drone's abdomen did not appear to be a sure or *specific* stimulus to "set off" the powerful muscles in the walls of the seminal vesicles and accessory glands. It must be evident that without the proper contraction of those muscles, and the consequent emptying of the stored spermatozoa and granular mucous together through the ejaculatory duct, no mechanical evagination of the penis, however complete, could be of any avail. Moreover, if successful mating is to be carried out (even in limited practice) while the queen and a properly selected drone are held in the correct position, there must be found some stimulus other than hand-pressure—a stimulus which will cause evagination of the male organ, and discharge of sperm-fluid by the contraction respectively of the abdominal wall muscles, and the muscular walls of the seminal vesicles and accessory mucous glands.

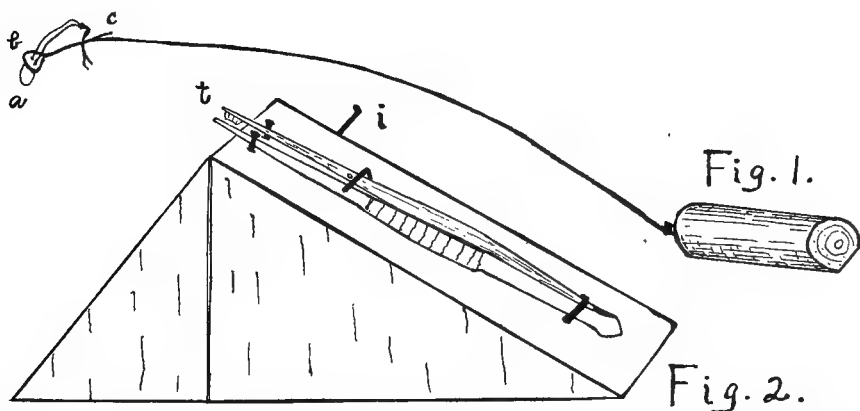


FIG. 1. Holder and fine elastic wire used in directing the movements of the drone and queen. The loop of the silk thread "a" passed around the body between the thorax and abdomen. The tiny metal disc "b" rested against the posterior dorsal portion of the thorax just beneath the wings. The V-shaped notch "c" served for fastening the thread.

FIG. 2. Forceps and block used in mating tests. t=thin metal blade on the lower edge of the right forceps prong—described in text. i=Pin used in certain mating tests.

PLATE I

Fig. 3. Reproductive organs of drone, side view, with the left-hand members of the paired organs removed. Pen = penis; Ac Gl = Accessory mucus gland; Ves = Vesicula seminalis; V. Det = Vas deferens; Tes = Testis; B = Bulb of penis (bean, Cheshire); tt = Chitin plates of bulb; wc = Wings of chitin plates of bulb; P = Parallel prongs of bulb-plates; uu = Doubly pinnate lobe; ww = Triangular dorsal plate of penis tube; xx = Quadrangular ventral plate of penis tube; zz = Membranous pouches—pneumophyses; vv = Cross striped chitinated ridges and creases; yy = Thin crinkle walled chamber of penis next to G SI, the genital slit, which is spread open. Ej D = Ejaculatory duct. The posterior end of the mucus gland is lifted up and a little to the right; also, the tips of the folded pneumophyses are lifted up. Otherwise, natural position.

Fig. 4. Reproductive organs of drone—penis extruded. Labeling same as in Fig. 3. o = outlet opening of ejaculatory duct; Ar. b = Bubbles of air. c = Cup of the everted bulb showing the surface of the elastic gelatinous-like thickening, ss.

Fig. 5. Vagina of queen (dorsal view) with sting and all other parts removed to show the entrances (ee) to the pouches or pockets of the Bursa copulatrix, and the entrance (ev) to the vagina. V = Vagina. Spm = Spermatheca or seminal receptacle turned a little to the left in order to show its duct (Spm d) which empties into the dorsal side of the vagina. Spm gl = tubular accessory glands of the spermatheca; Spm gl d = Duct of the Spm gl = empties into the upper end of the short spermathecal duct; Ov D = Ends of oviducts.

[illegible]

Pen

PLATE II

Fig. 6. Cross section of Accessory mucus gland near the anterior or blind end, showing the comparatively thin wall (w) of the gland in this region. m=granular mucus in the gland cavity shrunken away from the walls of the gland during process of fixing.

Fig. 7 and 8. Cross sections of accessory mucus gland made near the posterior end of the gland where the wall is quite muscular. a=Outer longitudinal muscle layer; b=Layer of circular muscles; c=Inner longitudinal muscle layer; d=Mucus gland cell layer; Fig. 8 shows the fringe of the mucus cell layer; g c=Mucus cavity or lumen of the gland.

Fig. 9. Cross section through the bulb of an extruded penis; Ac gl=The posterior end of an accessory mucus gland which was forced down into the penis, in this case, when that organ was extruded by hand pressure; ss=gelatinous-like thickening of the bulb; wc=Wings of chitin plates and tt=chitin plates of inverted bulb; Ej D=Cross section of ejaculatory duct (see Fig. 4). w=thin wall of bulb.

Fig. 10. Longitudinal section of a small part of the ejaculatory duct. hh=Hypodermal cell layer; ch=chitin; cl=canal.

Fig. 11. Small portion of the penis tube wall from "s" near the region of the cross striped area "vv" Fig. 4, or Fig. 12; h=hypodermal layer; ch=chitin; v=one of the chitinized bars of vv.

Fig. 12. Longitudinal dorso-ventral section through the extruded penis entire. Labeling as in Figs. 3 and 4. The Ej D is shown through almost its entire length. By examining successive sections, it could be followed right up to the base of the penis. Very little of the elastic gelatinous-like thickening of the bulb is shown since the Ej D slips over against one side of that thickening in the extruded penis as may be seen in cross section, Fig. 9. The lobes of the doubly pinnate lobed appendage (uu) are not everted here but show inside the appendage.

Fig. 13. Cross section of seminal vesicle. a=epithelial cell layer lining the vesicle cavity (vc); bm=Basement membrane of epithelial layer; b=inner layer of circular muscles. c=longitudinal muscle layer; d=Outer layer of circular muscle and connective fibres interwoven with tracheae (t).

Fig. 14. Part of cross section of seminal vesicle (1-8 objective) to show regular radiate arrangement of spermatozoa (SP) often found. Labeling same as in Fig. 13. The outer circular layer (d) was mostly torn away in this section.

Figs. 6 to 14 inclusive are photomicrographs.

Plate II

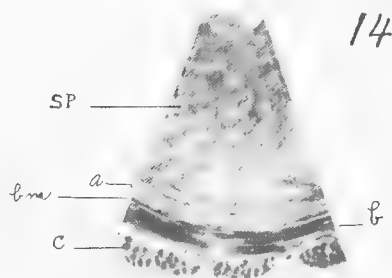
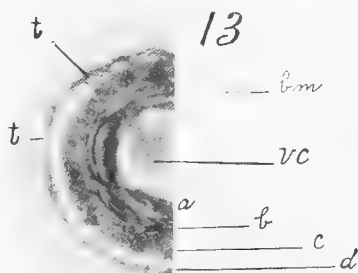
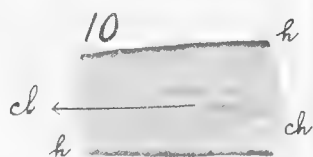
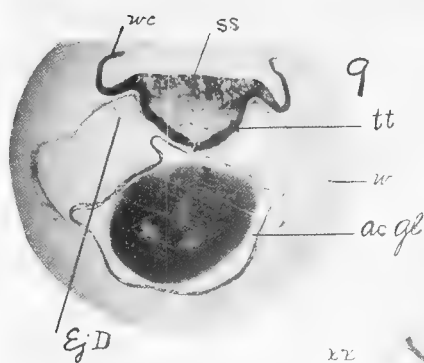
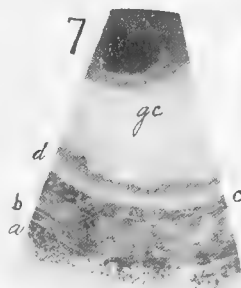
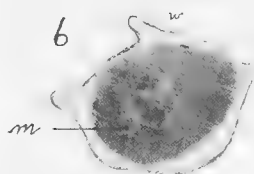


PLATE III

FIG. 15. Photograph of posterior end of abdomen of queen. Dcp and Vcp = Dorsal and ventral genito-anal cover plates; s = sting; Sn = sting notch.

FIG. 16. Drawing of posterior end of drone's abdomen showing the relative position which the copulatory organ must assume when extruded into the vagina instead of into the open air. In the latter case the drone organ extends backward (curving slightly dorsalward) from the tip of the abdomen in the position represented in Fig. 4. Dcp = Dorsal genito-anal cover plate.

FIG. 17. Cross section of oviduct (from queen just returned from mating) filled and greatly distended with coagulated granular sperm fluid.

FIG. 18. Small portion of section same as Fig. 17, but with higher power of microscope objective (J-6). Vw = Distended vaginal wall.

FIG. 19. Abdomen of newly mated queen received from Mr. Hunt. In this case the drone organ (Pen) had been torn off just proximad of the ventral cross-striped ridges (vv), and was pierced by the sting at "Stn." In the other newly mated queens the "bulb" was the only part of the drone organ left behind in the vagina.

FIG. 20. An enlargement of the tip of the abdomen shown in Fig. 19. Labeling same as in Fig. 19.

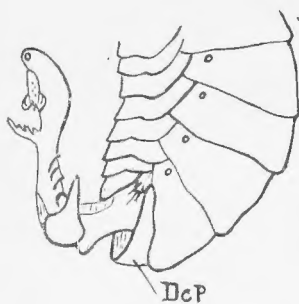
Figs. 17 and 18, photomicrographs.

Plate III

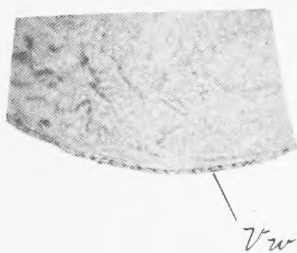
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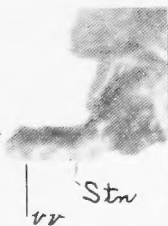
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Pen

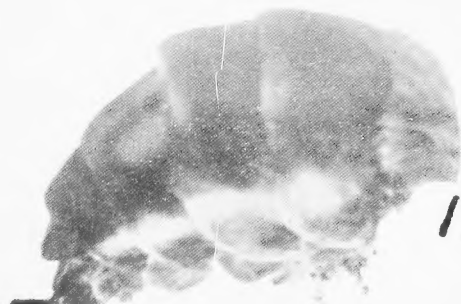
rr Stn

20



19

Pen rr Stn



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